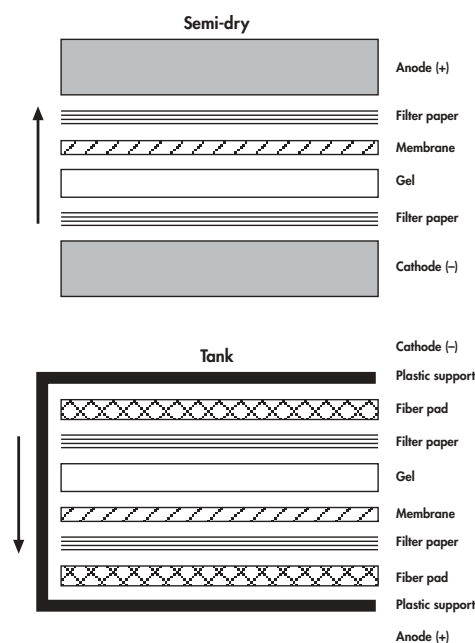




## Western blotting

Following electrophoresis, proteins in a polyacrylamide gel can be transferred to a positively charged membrane (e.g., Schleicher and Schuell BA85) in a buffer-tank-blotting apparatus or by semi-dry electroblotting as described below. With the semi-dry electroblotting method, the gel and membrane are sandwiched between two stacks of filter paper that have been pre-wet with transfer buffer. The membrane is placed near the anode (positively charged), and the gel is placed near the cathode (negatively charged). SDS-coated, negatively charged proteins are transferred to the membrane when an electric current is applied. With the tank-blotting method, a blotting cassette is submerged in a tank for blotting (Figure 4). Tank blotting can be performed over extended periods since the buffer capacity is far greater than that with semi-dry transfer systems. Results obtained with the tank-blotting method are typically better, with more efficient transfer, particularly of large proteins. Transfer efficiency can be checked by staining proteins on the membrane using Ponceau S (Protocol 8, page 79). Once transferred to the membrane, the proteins can be probed with epitope-specific antibodies or conjugates.

### Tank- and Semi-Dry Blotting Procedures



**Figure 4.** Schematic of tank- and semi-dry blotting methods. Arrows show direction of protein transfer.

## Protocol 7. Western transfer

### Materials

- ▶ Transfer apparatus
- ▶ Filter paper (e.g., Whatman 3MM)
- ▶ Positively-charged membrane (e.g., Schleicher and Schuell BA85)
- ▶ SDS polyacrylamide gel containing separated proteins (see Protocol 5, page 75)
- ▶ Transfer buffer (semi-dry or tank-blotting)

For buffer and reagent compositions, see “Western Transfer Buffers and Solutions”, page 97.

1. Cut 8 pieces of filter paper and a piece of membrane to the same size as the gel.
- Tip** To avoid contamination, always handle the filter paper, membrane, and gel with gloves.
2. Incubate membrane for 10 min in semi-dry or tank-blotting transfer buffer.
3. Soak filter paper in semi-dry or tank-blotting transfer buffer.

#### 4A. Semi-dry transfer:

Avoiding air bubbles, place 4 sheets of filter paper on the cathode (negative, usually black), followed by the gel, the membrane, 4 sheets of filter paper, and finally the anode (positive, usually red). See Figure 4.

#### 4B. Tank-blotting:

Avoiding air bubbles, place 4 sheets of filter paper on the fiber pad, followed by the gel, the membrane, 4 sheets of filter paper, and finally the second fiber pad (see Figure 4).

**Tip** Air bubbles may cause localized nontransfer of proteins. They can be removed by gently rolling a Pasteur pipet over each layer in the sandwich.

5. Carry out the transfer procedure. For current, voltage, and transfer times specific to your apparatus, consult the manufacturer’s instructions.

▶▶▶ protocol continues overleaf



### Protocol 7. Continued

**Tip** Time of transfer is dependent on the size of the proteins, percentage acrylamide, and gel thickness. Transfer efficiency should be monitored by staining (see below). The field strength required is determined by the surface area and thickness of the gel: 0.8 mA/cm<sup>2</sup> is a useful guide (1 h transfer).

6. After transfer, mark the orientation of the gel on the membrane.

### Protocol 8. Ponceau S staining

1. Incubate membrane in Ponceau S staining solution (see “Western Transfer Buffers and Solutions”, page 97) with gentle agitation for 2 min.
2. Destain in distilled water until bands are visible.

**Tip** Check that proteins of different sizes have been transferred uniformly to the membrane. Hydrophobic proteins may be more efficiently transferred by increasing the percentage of methanol in the transfer buffer.

3. Mark membrane using a suitable pen (i.e., one not containing water-soluble ink) or pencil, or cut as desired.

### Dot blots

Dot blotting is a simple, convenient method for detection of proteins in crude lysates or solutions without the need for separation by SDS-PAGE. This method is especially useful as a simple control because it avoids problems that may be due to the western transfer process. Any components that interfere with binding or bind nonspecifically, however, will not be spatially separated from the protein and will interfere with the intensity of signals. Suitable controls should always be employed to compensate for this.

### Protocol 9. Preparation of dot blots

#### Materials

- ▶ Nitrocellulose membrane (e.g., Schleicher and Schuell BA85)
- ▶ Protein samples
- ▶ Dilution buffer for native or denaturing conditions

For buffer and reagent compositions, see “Protein Dot-Blot Preparation Buffers”, page 98.

1. Dilute protein samples in buffer to final protein concentrations of 1–100 ng/μl.

**Tip** The protein of interest is diluted in dilution buffer for denaturing conditions, dilution buffer for native conditions, or another preferred buffer.

2. Apply 1 μl samples of diluted protein directly onto membrane. It is also possible to use crude cell lysate and apply 1 μl samples with an estimated concentration of 1–100 ng/μl protein.

**Note:** Under native conditions especially, the antibody epitope must be at least partially exposed to allow antibody binding. In most cases diluting the protein with buffer containing denaturing reagents will increase epitope exposure and give better results.

**Tip** To differentiate between nonspecific and positive signals, an extra sample containing 1 μl of a cell extract of the host strain without plasmid (or other suitable control) should also be applied to the membrane and treated together with the protein of interest.

3. After applying the samples, the membrane should be dried for a short time at room temperature before proceeding with the detection process.

**Tip** For larger sample volumes, suitable equipment is available from several suppliers.

4. Proceed with immunodetection (Protocol 10 or 11, page 81 or 82, respectively).

### Protocol 8

### Protocol 9